

WHAT IS CLAIMED IS:

1. A rapid analytical method for detection of A β amyloid formation in a biological fluid which comprises:

(a) preparing a first set of reaction mixtures comprising neat biological fluid from a control human subject, and serial dilutions of the same made in aqueous buffer or physiological solution;

(b) preparing a second set of reaction mixtures comprising neat biological fluid from a human patient suspected of amyloidosis, and serial dilutions of the same made in aqueous buffer or physiological solution;

(c) adding an equal amount of A β peptide comprising at least amino acids 6 to 28 of A β to each serial dilution sample;

(d) contacting each of the first and the second set of reaction mixtures with an amount greater than 300 nM of a heavy metal cation capable of binding to an A β peptide comprising at least amino acids 6 to 28 of A β ;

(e) centrifuging each of the first and the second sets of reaction mixtures to give a first and a second set of pellets, respectively; and

(f) comparing the amount of amyloid in the first and the second set of pellets and thereby detecting excessive A β amyloid formation in the biological fluid from the human patient suspected of amyloidosis.

2. A rapid analytical method for detection of A β amyloid formation in a biological fluid as claimed in claim 1, wherein said biological fluid is CSF.

3. A rapid analytical method for detection of A β amyloid formation in a biological fluid as claimed in claim 2, wherein in step (c), said heavy metal cation capable of binding to an A β peptide comprising at least amino acids 6 to 28 of A β is zinc.

4. A method for determining whether a compound inhibits formation of A β amyloid which comprises:

(a) pre-filtering an aqueous buffer solution of A β peptide, which comprises at least the region in the A β peptide from amino acid number 6 to 28 to give a first filtrate;

(b) measuring the amount of A β peptide in the first filtrate obtained in step (a);

(c) contacting the first filtrate obtained in step (a) with a heavy metal cation capable of binding to the peptide comprising at least amino acids 6 to 28 of A β to give a reaction mixture;

(d) contacting the reaction mixture obtained in step (c) with a candidate anti-amyloidotic agent;

(e) filtering the reaction mixture obtained in step (d) to give a second filtrate; and

(f) comparing the amount of A β peptide in the second filtrate with the amount of A β peptide in the first filtrate, thereby determining whether the candidate compound inhibits formation of A β amyloid.

5. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 4, wherein the heavy metal cation is selected from the group consisting of metalochloride salts of zinc, copper, and mercury.

6. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 4, wherein the heavy metal cation is zinc chloride.

7. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 6, wherein said A β peptide is selected from the group consisting of A β_{1-39} , A β_{1-40} , A β_{1-41} , A β_{1-42} , and A β_{1-43} .

8. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 6, wherein said A β peptide is A β_{1-40} .

9. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 4, wherein the pH of the reaction mixtures are between 6.8 to 7.8.

10. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 4, wherein the pH of the reaction mixtures are about 7.4.

11. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 4, wherein the concentration of the A β peptide is about 0.8 μ M.

12. A method for determining whether a compound inhibits formation of A β amyloid which comprises:

(a) assembling a first and a second reaction mixture, wherein each reaction mixture comprises an equal amount of a pre-filtered A β peptide solution, which comprises at least the region in the A β peptide from amino acid number 6 to 28, and an aqueous buffer or physiological solution;

(b) contacting each of the first and the second reaction mixtures with an equal amount of a candidate anti-amyloidotic agent;

(c) contacting the first reaction mixture with a heavy metal cation capable of binding to the peptide comprising at least amino acids 6 to 28 of A β ;

(d) contacting the second reaction mixture with EDTA; and

(e) comparing the amount of amyloid formed in the first reaction mixture with that in the second reaction mixture, thereby determining whether the candidate compound inhibits the formation of A β amyloid.

13. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 12, wherein the concentration of A β peptide in the reaction mixture is about 0.8 μ M.

14. A method for determining whether a compound inhibits formation of A_β amyloid as claimed in claim 12, wherein step (d) comprises the steps of:

(i) centrifuging the first and the second reaction mixtures, so that the soluble A β peptides are separated from the insoluble amyloid and a pellet is formed; and

(ii) comparing the amount of soluble A β peptide in the first reaction mixture with the soluble A β peptide in the second reaction mixture, thereby determining effectiveness of the candidate anti-amyloidotic agent.

15. A method for determining whether a compound inhibits formation of A_β amyloid as claimed in claim 12, wherein the pH of the reaction mixtures are about 6.8 to 7.8.

16. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 12, wherein the pH of the reaction mixtures are about 7.4.

17. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 14, wherein in step (ii), said pellets are stained with an amyloid-staining dye.

18. A method for determining whether a compound inhibits formation of A_β amyloid as claimed in claim 14, wherein said heavy metal cation is selected from the group consisting of salts of zinc, copper, and mercury.

19. A method for determining whether a compound inhibits formation of A_β amyloid as claimed in claim 14, wherein said heavy metal cation is a zinc salt

20. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 12, wherein step (a), the A β peptide solution

is prefiltered before assembling said first and second reaction mixtures; and wherein step (d) comprises the steps of:

(i) filtering the first and the second reaction mixtures, separately, and

(ii) comparing the amount of A β peptide in the filtrate, thereby determining effectiveness of the candidate anti-amyloidotic agent.

21. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 20, wherein step (d)(ii) comprises the step of measuring the fraction of A β peptide in the filtrate by calculating ratio of the filtrate OD₂₁₄ relative to the OD₂₁₄ of the prefiltered A β peptide solution at step (a), thereby determining whether the compound inhibits formation of A β amyloid.

22. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 21, wherein said A β peptide is selected from the group consisting of A β ₁₋₃₉, A β ₁₋₄₀, A β ₁₋₄₁, A β ₁₋₄₂, and A β ₁₋₄₃.

23. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 21, wherein said A β peptide is A β ₁₋₄₀.

24. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 21, wherein all filtering is done using filters with a pore size that allows passage of the soluble A β peptide used in the reaction mixtures but does not allow passage of amyloid.

25. A method for determining whether a compound inhibits formation of A β amyloid which comprises:

(a) assembling a first and a second reaction mixture, wherein each reaction mixture comprises an equal amount of a prefiltered A β peptide

solution, which contains at least the region in the A β peptide from amino acid number 6 to 28, and an aqueous buffer or physiological solution;

(b) contacting each of the first and the second reaction mixtures with an equal amount of a candidate anti-amyloidotic agent;

(c) contacting only the first reaction mixture with a heavy metal cation capable of binding to the peptide comprising at least amino acids 6 to 28 of A β ; and

(d) comparing the amount of amyloid formed in the first reaction mixture with that in the second reaction mixture, thereby determining whether the compound inhibits formation of A β amyloid.

26. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 25, wherein the concentration of A β peptide in the reaction mixture is about 0.8 μ M.

27. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 25, wherein step (d) comprises the steps of:

(i) filtering the first and the second reaction mixtures, separately, through filters with a pore size that allows passage of the soluble A β peptide used in the reaction mixtures but does not allow passage of amyloid; and

(ii) comparing the amount of amyloid accumulated at step (i) on the filters, thereby determining effectiveness of the candidate anti-amyloidotic agent.

28. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 12, wherein said physiological solution is CSF.

29. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 25, wherein said physiological solution is CSF.

30. A method for determining whether a compound inhibits formation of A β amyloid which comprises:

- (a) establishing a first and a second cell culture comprising a cell line which expresses at least a human A β peptide comprising at least the region of the A β peptide from amino acid number 6 to 28;
- (b) contacting equal concentrations of zinc to each cell culture;
- (c) contacting the first cell culture with the candidate agent, and contacting the second cell culture with a heavy metal chelating agent; and
- (d) comparing the amount of amyloid and zinc-induced A β aggregates in each cell culture, thereby determining effectiveness of the candidate anti-amyloidotic agent.

31. A method for determining whether a compound inhibits formation of A β amyloid as claimed in claim 30, wherein said heavy metal chelating agent is EDTA or Desferrioxamine.

32. A method for determining whether a compound inhibits formation of A β amyloid which comprises:

- (a) establishing a first and a second cell culture comprising a cell line which expresses at least a human A β peptide comprising at least the region of the A β peptide from amino acid number 6 to 28;
- (b) contacting the first cell culture with zinc to give a first reaction mixture;
- (c) contacting the first reaction mixture and the second cell culture with the candidate agent; and
- (d) comparing the amount of amyloid and zinc-induced A β aggregates in each cell culture, thereby determining effectiveness of the candidate anti-amyloidotic agent.

33. A kit for determining whether a compound inhibits formation of A β amyloid which comprises a carrier means being compartmentalized to receive in close confinement therein one or more container means wherein

- (a) the first container means contains a peptide comprising at least the region of the A β peptide from amino acid number 6 to 28; and
- (b) a second container means contains a heavy metal cation.

34. The kit of claim 33, wherein said A β peptide is present as a solution in an aqueous buffer or a physiological solution, at a concentration above about 10 μ M.

35. The kit of claim 34, wherein said concentration is about 10 to about 25 μ M.

36. The kit of claim 33, wherein said A β peptide is present in lyophilized form.

37. The kit of claim 33, wherein said heavy metal cation is present as a metallochloride solution, at a concentration above about 300 nM.

38. The kit of claim 37, wherein said concentration is about 25 μ M.

39. The kit of claim 37, wherein said heavy metal cation is zinc.

40. The kit of claim 38, wherein said heavy metal cation is zinc.

41. The kit of claim 33, further comprising

- (c) one or more container means containing standard solutions of chelators of heavy metal cations.

42. The kit of claim 41, further comprising
 - (d) one or more container means containing standard solutions of amyloid-staining dyes.